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Activation of rat splenic macrophage and lymphocyte functions by fumonisin B_1

Mary A. Dombrink-Kurtzman [a,*], Ricardo Gomez-Flores [b], Richard J. Weber [b]

[a] *Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, 1815 N. University Street, Peoria, IL 61604-3902 USA*

[b] *Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL, USA*

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Abstract

Fumonisins represent a family of toxic, structurally related metabolites produced by fungi that are found in corn worldwide. We investigated the effects of the mycotoxin, fumonisin B_1, on rat splenic macrophage and lymphocyte functions. Pretreatment (24 h) of resident macrophages with fumonisin B_1 (1, 10, and 100 μg/ml) significantly ($p < 0.01$) stimulated nitric oxide production (0.48, 2.60, and 4.40 nmol nitrite/well, respectively), compared with the response of untreated macrophages (no nitrite detected), after 72 h of culture. Fumonisin B_1 (1 and 10 μg/ml) and IFN-γ acted in an additive manner to activate nitric oxide production. The response of IFN-γ (50 U/ml)-activated macrophages (1.68 nmol nitrite/well) was potentiated (3.52, 4.96, and 4.44 nmol nitrite/well) by fumonisin B_1 (1, 10, and 100 μg/ml, respectively). In addition, fumonisin B_1 significantly ($p < 0.05$) potentiated Con A (1.25 to 5 μg/ml) (1.46- to 2.62-fold increases)- and antiTCR, IL-2 or antiTCR + IL-2 (1.72- to 2.60-fold increases)-induced proliferation of splenic cells in the presence of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (NMA). These results show two distinct and separate effects of fumonisin B_1: it induces nitric oxide production by macrophages and it stimulates T cell proliferation. Published by Elsevier Science B.V.

Keywords: Fumonisin B_1; Macrophage activation; Nitric oxide; T cell proliferation; Cell growth

1. Introduction

Fumonisins represent a family of toxic, structurally related fungal metabolites, known as mycotoxins. The major fumonisin, fumonisin B_1, is usually present as 70% of the total fumonisins detected in corn (maize). Fumonisins are produced by the fungi, *Fusarium moniliforme* and *F. proliferatum*, and have been found in corn all over the world, as well as in processed corn products and animal feeds (reviewed by Nelson et al., 1993). Even when there are no visible signs of fungal contamination, corn often contains low levels of fumonisins. Although the structure of fumonisin B_1 (Fig. 1) was only elucidated in 1988 (Bezuidenhout et al., 1988), the toxic effects associated with moldy corn when fed to

Abbreviations: Con A, concanavalin A; IL-2, interleukin-2; LPS, lipopolysaccharide; NMA, N^G-monomethyl-L-arginine; antiTCR, IgG_1 monoclonal antibody to rat T cell receptor$_{\alpha\beta}$

* Corresponding author. Tel.: +1-309-681-6254; fax: +1-309-681-6686.

E-mail address: dombrink@mail.ncaur.usda.gov (M.A. Dombrink-Kurtzman).

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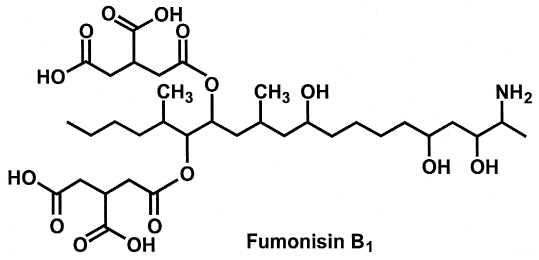

Fig. 1. Structure of fumonisin B_1.

horses have been known for almost a century (Butler, 1902). Fumonisin has been identified as the causative agent of equine leukoencephalomalacia (ELEM) (Marasas et al., 1988), porcine pulmonary disease (Harrison et al., 1990), and hepatotoxicity and nephrotoxicity in rats (Voss et al., 1993) and has been linked epidemiologically with human esophageal cancer in the Transkei area, South Africa (Rheeder et al., 1992) and in Linxian County, Henan Province, China (Chu and Li, 1994).

Research by the laboratories of Merrill and Riley (Wang et al., 1991) has established that fumonisin disrupts de novo sphingolipid biosynthesis by interfering with sphinganine (sphingosine) *N*-acyltransferase (ceramide synthase). Following both in vivo and in vitro exposures to fumonisin B_1 in a variety of cells (hepatocytes, kidney cells, neurons, and fibroblasts), elevated levels of sphinganine accumulates, biosynthesis of ceramides and complex sphingolipids is blocked, and increases in the sphinganine to sphingosine ratio serve as a biomarker for fumonisin exposure (reviewed by Merrill et al., 1996). Fumonisin B_1 can also induce apoptosis in CV-1 cells (African green monkey kidney cells) and cell cycle arrest in the G_1 phase (Wang et al., 1996). Although little is known about the effects of fumonisin B_1 on immune functions, a recent report indicated that macrophages from mice injected subcutaneously with fumonisin B_1 showed increased production of tumor necrosis factor-α (TNF-α) (Dugyala et al., 1998).

Because of the important role that macrophages play in host defense, the first objective of the present study was to evaluate the effect of fumonisin B_1 on both resident and interferon-γ (IFN-γ)-activated macrophage function. A second objective was to examine the effect of fumonisin B_1 on lymphocyte proliferation. Two distinct effects are shown: (1) fumonisin B_1 stimulated and potentiated NO production by resident and IFN-γ-activated macrophages, respectively; (2) fumonisin B_1 stimulated T cell proliferation of untreated and mitogen-treated splenic cells when an inhibitor of nitric oxide synthase was present.

2. Materials and methods

2.1. Reagents and culture media

Rat IFN-γ (specific activity of 4×10^6 U/mg), penicillin–streptomycin and trypsin–EDTA solutions, and RPMI 1640 and AIM-V media were obtained from Life Technologies (Grand Island, NY). Fetal bovine serum (FBS), Con A, red blood cell lysing buffer, PBS, and N^G-monomethyl-L-arginine (NMA) were purchased from Sigma (St. Louis, MO). Fumonisin B_1 (> 95% purity) was obtained from Dr. Robert M. Eppley, US Food and Drug Administration, Washington, DC. Fumonisin B_1 was tested by the kinetic chromogenic *Limulus* amoebocyte assay (performed by Charles River Endosafe, Charleston, SC) for the presence of endotoxin; 0.0457 ng of endotoxin/10 μg of fumonisin B_1 was detected. Monoclonal antibodies (IgG_1) to T cell receptor$_{\alpha\beta}$ (antiTCR) were obtained from Harlan Bioproducts for Science (Indianapolis, IN).

2.2. Animals

Sprague–Dawley male rats (200–220 g) were purchased from Harlan Sprague–Dawley (Indianapolis, IN). They were given water and food ad libitum.

2.3. Splenic macrophage preparation and culture

Spleens were removed immediately after rat sacrifice. A single cell-suspension was prepared by disrupting the organ in RPMI 1640 medium as previously reported (Nowak et al., 1998). The cell suspension was washed three times in this medium, resuspended and adjusted at 5×10^6 cells/ml in AIM-V medium (containing 0.5% penicillin–streptomycin solution). Because serum has been reported to potentiate murine macrophage activation (Chen et

al., 1994), the culture medium was changed at this step to AIM-V medium, a serum-free medium capable of supporting cell culture (Kaldjian et al., 1992). One hundred microliters of the splenic cell suspension were plated in triplicates in flat-bottomed 96-well plates (Becton Dickinson, Cockeysville, MD) for 2 h at 37°C. Non-adherent cells were removed, and remaining red blood cells were lysed by adding 50 μl of red blood cell lysing buffer, followed by 50 μl of AIM-V medium. Adherent cells (about 10% of the input cells or 5×10^4 cells/well) were then incubated overnight in 200 μl AIM-V medium with or without IFN-γ (15.6 to 500 U/ml), and with or without various concentrations of fumonisin B_1 (0, 0.01, 0.1, 1, 10, and 100 μg/ml in AIM-V medium) in the presence or absence of 50 U/ml of IFN-γ. After overnight incubation, cell monolayers were washed once in AIM-V medium. The final adherent cell monolayer consisted of 95–99% macrophages as judged by Giemsa's stain procedures.

2.4. Nitrite determination

Accumulation of nitrite in the supernatants of macrophage cultures was used as an indicator of nitric oxide production by resident or activated cells (Gomez-Flores et al., 1997). Splenic untreated macrophages, IFN-γ-primed (24 h stimulation) macrophages, and macrophages stimulated for 24 h with fumonisin B_1 in the presence or absence of IFN-γ (50 U/ml) were washed once with AIM-V medium and incubated in a total volume of 200 μl AIM-V medium per well for 72 h. To determine the effect of NMA on FB_1-induced nitric oxide production by splenic macrophages, adherent cells were also incubated with or without various concentrations of NMA (50 to 400 μg/ml) for 72 h. After incubation, supernatants were obtained, and nitrite levels were determined with the Griess reagent as reported previously (Gomez-Flores et al., 1997), using $NaNO_2$ as standard. Optical densities at 540 nm were then determined in a microplate reader (Molecular Devices, Palo Alto, CA).

2.5. T cell proliferation assay

T cell proliferation was determined by [^{3}H]-thymidine uptake as previously reported (Nowak et al., 1998). Immediately after rat sacrifice, a single-cell suspension was prepared from spleen, as described above, and adjusted to 5×10^6 cells/ml. Cell suspensions (100 μl) were added to round-bottomed 96-well plates (Becton Dickinson) containing triplicate cultures (100 μl) of AIM-V medium (unstimulated control) or the mitogen Con A at submaximal and maximal concentrations (1.25, 2.5 and 5 μg/ml), antiTCR (5 μg/ml), IL-2 [5% of a 24 h-conditioned medium from Con A (2.5 μg/ml)-stimulated splenic cells], or antiTCR + IL-2, to stimulate lymphoproliferation, in the presence or absence of fumonisin B_1 (0, 0.1, 1, 10, and 100 μg/ml) and/or NMA (100 μg/ml). After incubation for 44 h at 37°C in an atmosphere of 5% CO_2–95% air, [^{3}H]-thymidine (6.7 Ci/mmol, ICN Pharmaceuticals) was added (1 μCi/10 μl/well), and cultures were incubated for an additional 4 h. Because nitric oxide is a well known suppressor of T cell proliferation (Albina et al., 1991), splenic cells were also treated with NMA to study the potential antagonistic effect of fumonisin B_1-induced nitric oxide on splenic T cell proliferation. To determine the experimental NMA dose for T cell proliferation experiments, splenic cells were incubated with NMA (50 to 400 μg/ml) in the presence or absence of Con A (1.25 to 5 μg/ml) for 48 h. Cell cultures were then harvested with a semiautomatic cell harvester (Tomtec), and cell-incorporated radioactivity was determined by liquid scintillation spectrophotometry using a Microbeta Plus liquid scintillation counter (Wallac) with a counting efficiency for tritium of 35%. Proliferative responses of splenic lymphocytes to maximal and submaximal concentrations of Con A were used for data analysis. T cell specific proliferative responses were evaluated by using antiTCR or IL-2 alone, or in combination, as co-stimulating signals for lymphocyte proliferation.

2.6. Statistical analysis

The results were expressed as mean ± SEM of three independent experiments. Results are presented either as fold increase/decrease values compared with the untreated control or as mean concentration (nmol/well). Statistical significance was determined by Student's *t*-test, comparing the experimental group to the control group at each level of Con A, antiTCR, IL-2 or antiTCR + IL-2 for T cell analysis.

3. Results

3.1. Effect of fumonisin B_1 on nitric oxide production by splenic macrophages

Fumonisin B_1 was observed to activate nitric oxide production by splenic macrophages, and to potentiate IFN-γ-induced nitric oxide release. We found that pretreatment (24 h) with fumonisin B_1 (1, 10, and 100 μg/ml) significantly ($p < 0.01$) stimulated nitric oxide production by resident macrophages (0.48, 2.60, and 4.40 nmol nitrite/well, respectively), compared with the response of untreated macrophages (0 nmol nitrite/well), and potentiated that of IFN-γ (50 U/ml)-activated macrophages (2.10-, 2.95-, and 2.64-fold increases, respectively), compared with the response of IFN-γ (50 U/ml)-treated macrophages (1.68 nmol nitrite/well), after 72 h of culture (Fig. 2). Moreover, macrophages treated with IFN-γ alone at doses ranging from 15.6 to 500 U/ml, produced 1.00–2.48 nmol nitrite/well, compared with the response of untreated macrophages (0 nmol nitrite/well) (data not shown). Induction of nitric oxide by IFN-γ, at all doses tested (15.6 to 500 U/ml), was lower than the effect of 10 and 100 μg/ml of fumonisin B_1 (Fig. 2). In addition, following co-stimulation with IFN-γ (50 U/ml), fumonisin B_1 (1 and 10 μg/ml) was shown to possess an additive effect to activate nitric oxide production by macrophages (Fig. 2).

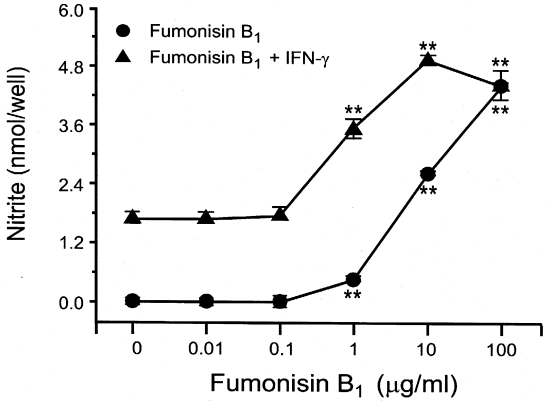

Fig. 2. Effect of fumonisin B_1 and/or IFN-γ on nitric oxide production by rat splenic macrophages. Macrophages were treated with fumonisin B_1 (0.01 to 100 μg/ml) in the presence or absence of IFN-γ (50 U/ml) for 24 h, after which macrophage monolayers were washed and incubated with fresh medium for an additional 72 h; nitrite was determined as explained in the text. Data represent mean ± SEM of triplicates from three independent experiments. $^{**}p < 0.01$ as compared with fumonisin B_1-untreated macrophage response.

3.2. Effect of NMA on fumonisin B_1-induced nitric oxide production by splenic macrophages

A positive correlation was observed between increasing concentrations of fumonisin B_1 (0.1–100 μg/ml) and nitrite levels detected at each level of NMA (0, 50, 100 and 400 μg/ml) (data not shown). Nitric oxide production decreased for all doses of fumonisin B_1 (0.1–100 μg/ml) as the concentration of NMA (50–400 μg/ml) increased, demonstrating that NMA inhibited NO induction by fumonisin B_1. Data were compared to the response of fumonisin B_1- and NMA-untreated cells (0 nmol nitrite/well). The highest nitrite levels occurred at fumonisin B_1 concentrations of 1, 10, and 100 μg/ml in the absence of NMA.

3.3. Effect of NMA on splenic cell proliferation

To determine the experimental NMA dose for T cell proliferation experiments, splenic cells were incubated with NMA (50 to 400 μg/ml) in the presence or absence of Con A. The response of NMA-treated and NMA-untreated splenic lymphocytes were compared at each dose of Con A. NMA (200 and 400 μg/ml) significantly ($p < 0.05$) decreased the splenic cell proliferative response to Con A at all levels tested. In contrast, lower doses of NMA (50 and 100 μg/ml) did not affect splenic cell proliferation. Therefore, we selected 100 μg/ml of NMA for use in subsequent experiments dealing with T cell proliferation.

3.4. Effect of fumonisin B_1 on splenic T cell proliferative response to Con A

In the absence of NMA, fumonisin B_1 (at its highest concentration, 100 μg/ml) enhanced proliferation significantly ($p < 0.05$) only with the highest concentration of Con A (5 μg/ml) (Table 1). Fu-

Table 1
Effect of fumonisin B_1 on splenic lymphocyte proliferation in the presence or absence of NMA

Fumonisin B_1 (μg/ml)	Con A (μg/ml)			
	0	1.25	2.5	5
− NMA				
0.1	0.93 ± 0.22[a]	0.85 ± 0.17	0.99 ± 0.16	1.10 ± 0.23
1	1.10 ± 0.12	0.92 ± 0.25	0.90 ± 0.30	0.86 ± 0.44
10	1.18 ± 0.12	1.16 ± 0.15	1.25 ± 0.18	1.22 ± 0.33
100	0.89 ± 0.25	1.04 ± 0.08	1.22 ± 0.08	1.79 ± 0.05 *
+ NMA (100 μg / ml)				
0.1	0.90 ± 0.08	0.91 ± 0.03	1.02 ± 0.01	1.04 ± 0.16
1	1.10 ± 0.04	1.16 ± 0.03	1.20 ± 0.06	1.13 ± 0.24
10	1.30 ± 0.23	1.38 ± 0.30	1.81 ± 0.06 *,b	2.09 ± 0.12 *,b
100	1.32 ± 0.09 *,c	1.46 ± 0.11 *,c	1.95 ± 0.37 *,c	2.62 ± 0.04 *,c

* $p < 0.05$ compared with the response of fumonisin-untreated cells at each dose of Con A.

[a] Data represent mean fold increase/decrease value ± SEM of three independent experiments. Fold increase/decrease values are the ratios between the response of fumonisin-treated and fumonisin-untreated splenic lymphocytes at each dose of Con A. Fold values lower or higher than 1.0 indicate suppression or enhancement, respectively, of splenic cell proliferative responses compared with the response of fumonisin-untreated cells, while a value of 1.0 indicates no effect. Splenic cell proliferative responses of NMA-untreated/fumonisin-untreated cells (controls) to Con A (0, 1.25, 2.5, and 5 μg/ml) were $2.68 \pm 0.20 \times 10^3$, $32.15 \pm 1.44 \times 10^3$, $45.87 \pm 2.55 \times 10^3$, and $41.76 \pm 1.84 \times 10^3$ cpm, respectively.

[b] $p < 0.05$ compared with the response of splenic cells not treated with NMA.

[c] $p < 0.02$ compared with the response of splenic cells not treated with NMA.

monisin B_1 did not affect the proliferative capacity of resident untreated splenic cells or cells treated with Con A (1.25 and 2.5 μg/ml). However, in the presence of NMA, fumonisin B_1 produced a greater

Table 2
Effect of fumonisin B_1 on splenic lymphocyte proliferation in the presence or absence of NMA

Fumonisin B_1 (μg/ml)	Untreated	antiTCR	antiTCR + IL-2	IL-2
− NMA				
0.1	0.93 ± 0.22[a]	1.45 ± 0.36	1.35 ± 0.36	1.20 ± 0.23
1	1.10 ± 0.12	1.46 ± 0.48	1.28 ± 0.17	1.03 ± 0.44
10	1.18 ± 0.12	1.29 ± 0.30	1.22 ± 0.17	0.84 ± 0.33
100	0.89 ± 0.25	0.88 ± 0.10	1.04 ± 0.05	1.16 ± 0.05
+ NMA (100 μg / ml)				
0.1	0.90 ± 0.08	ND	ND	ND
1	1.10 ± 0.04	ND	ND	ND
10	1.30 ± 0.23	1.94 ± 0.06 *,b	2.60 ± 0.12 *,c	1.80 ± 0.12 *,b
100	1.32 ± 0.09 *,d	1.94 ± 0.22 *,b	2.41 ± 0.06 *,c	1.72 ± 0.04 *,b

ND, not done.

* $p < 0.05$ compared with the response of fumonisin-untreated cells at each dose treatment.

[a] Data represent mean fold increase/decrease value ± SEM of three independent experiments. Fold increase/decrease values are the ratios between the response of fumonisin-treated and fumonisin-untreated splenic lymphocytes at each treatment. Fold values lower or higher than 1.0 indicate suppression or enhancement, respectively, of splenic cell proliferative responses compared with the response of fumonisin-untreated cells, while a value of 1.0 indicates no effect.

[b] $p < 0.02$ compared with the response of splenic cells not treated with NMA.

[c] $p < 0.005$ compared with the response of splenic cells not treated with NMA.

[d] $p < 0.05$ compared with the response of splenic cells not treated with NMA.

enhancement of background and Con A-induced proliferation. The use of NMA induced a significant ($p < 0.05$) proliferative response of Con A (2.5 and 5 μg/ml)-treated splenic cells to fumonisin B_1 (10 μg/ml), and significantly ($p < 0.05$) enhanced the proliferative response of untreated and Con A (1.25–5 μg/ml)-treated splenic cells to fumonisin B_1 (100 μg/ml) (Table 1).

3.5. Effect of fumonisin B_1 on splenic T cell proliferative response to antiTCR and / or IL-2

In the absence of NMA, fumonisin B_1 at all concentrations tested (1 to 100 μg/ml) did not affect the untreated resident or antiTCR- and/or IL-2-induced splenic cell proliferative response (Table 2). The use of NMA, however, significantly ($p < 0.05$) enhanced the proliferative response of untreated and antiTCR- and/or IL-2-treated splenic cells to fumonisin B_1 (100 μg/ml, and 10 and 100 μg/ml of fumonisin B_1, respectively) (Table 2). Results indicate that NO induction by fumonisin B_1 inhibited the ability of fumonisin B_1 to stimulate T cell proliferation.

4. Discussion

F. moniliforme and *F. proliferatum*, fungi capable of producing fumonisin, are ubiquitous in nature and occur naturally in corn and sorghum. Environmental conditions, such as drought stress, favor the production of fumonisins in developing grain. Poor storage conditions can also contribute to the continued growth of the fungi with additional accumulation of mycotoxins. Efforts to understand fumonisin's mechanism of action have been undertaken to ensure increased safety of both food and animal feed and a significant reduction in human health costs.

The ability of fumonisin B_1 to induce production of NO in both rat peritoneal (data not shown) and splenic macrophages (Fig. 2) suggests that macrophage activation may be involved with the toxic effects associated with fumonisin exposure. NO is produced in endothelial cells and macrophages by a family of isoenzymes known as nitric oxide synthases (NOSs) (Stuehr and Marletta, 1985; Hibbs et al., 1987; Olken and Marletta, 1993). Inducible nitric oxide synthase (iNOS) is induced in macrophages during activation. NO can act as a mediator of a variety of biological effects, among which are suppression of macrophage functions, tumor cytotoxicity, vasodilatation and inhibition of platelet aggregation. NO is a well known suppressor of T cell proliferation (Albina et al., 1991). Recent studies have demonstrated that low levels of NO can inhibit the proliferation of T-helper 1 and 2 lymphocytes without reduction in cytokine secretion (van der Veen et al., 1999). Both DNA deamination reactions and DNA strand breaks have been observed in vitro in response to NO (Wink et al., 1991). Increased iNOS expression has been associated with human esophageal squamous cell carcinomas (Tanaka et al., 1999), the type of esophageal cancer linked epidemiologically with consumption of fumonisin-contaminated corn (Rheeder et al., 1992; Chu and Li, 1994).

We have examined the effect fumonisin has on cultured macrophages because of the important role that macrophages play in host defense. Using rat splenic macrophages and serum-free medium, we observed that pretreatment (24 h) with fumonisin B_1 (10 to 100 μg/ml) significantly ($p < 0.01$) enhanced NO production by untreated macrophages after 72 h of culture (Fig. 2). These findings represent the first report of NO production induced by fumonisin B_1 in the absence of LPS. Stimulation of LPS-dependent NO production by fumonisin B_1 has been reported in a murine macrophage cell line (RAW 264.7) using a serum-containing medium, but NO release was not detected by fumonisin B_1 treatment in the absence of LPS (Rotter and Oh, 1996). We utilized rodent cells to demonstrate macrophage-derived NO. The high output of NO by iNOS appears to be restricted to macrophages of certain species. Induction of iNOS results in sustained release of NO at levels higher than is associated with the constitutive enzymes. There is no evidence that iNOS protein is expressed without an inductive event in macrophages and most other cells (MacMicking et al., 1997).

The conventional protocol for determining NO production is to measure nitrite accumulation, based

on incubating the cells with an activator for 24–36 h. In our experiments, however, we have incubated for 72 h because we had observed that fumonisin B_1 had a potent and lasting effect on macrophage activation, even after only overnight stimulation with this agent. This may indicate either that fumonisin B_1 binds strongly to surface receptors on macrophages, constantly stimulating them to produce nitric oxide, or that fumonisin B_1 may have washed off, but its effects were already set in motion.

Our results reported here have demonstrated that pretreatment with fumonisin B_1 significantly enhanced NO release by both resident untreated and IFN-γ-activated macrophages (Fig. 2), suggesting the potential involvement of fumonisin B_1 in promoting an inflammatory response. Haschek et al. (1992) had previously proposed that pulmonary intravascular macrophages (PIM) are involved in outbreaks of porcine pulmonary edema associated with fumonisin-containing feed by triggering the release of inflammatory mediators. Additionally, macrophage function may be compromised because NO produced by macrophages can mediate cytostatic action toward both target cells and the macrophages themselves (data not shown), when greater amounts of NO are produced (Albina and Reichner, 1998).

Various hematologic effects have been observed following exposure to fumonisin. Single dose of fumonisin B_1 (25 mg/kg administered subcutaneously) to BALB/c mice caused a dose-related increase in TNF-α and a significant increase in reticulocytes, progenitors of red blood cells (Dugyala et al., 1998). Abnormally shaped red cells resembling early stages of erythroblasts (Dombrink-Kurtzman et al., 1993) and significant decreases in red blood cell counts, hemoglobin and white cell counts (Javed et al., 1995) have been observed in peripheral blood of broiler chicks given feed amended with *F. proliferatum* culture material containing fumonisin B_1, fumonisin B_2, and moniliformin.

Inhibition of proliferation has been observed with turkey peripheral blood lymphocytes (macrophages had not been completely removed) that had been exposed in vitro to fumonisin B_1 or fumonisin B_2, whereas control lymphocytes were able to proliferate (Dombrink-Kurtzman et al., 1994). We have also observed thymic lymphocyte proliferation mediated by fumonisin B_1 (data not shown); however, the inhibitory effect of nitric oxide on lymphoproliferation is naturally observed in splenic cell populations, which include both macrophages and lymphocytes. Results described here indicate that nitric oxide was associated with abrogating the effects of fumonisin B_1 on lymphocyte proliferation because the use of the NOS inhibitor, NMA, significantly potentiated the fumonisin B_1-mediated splenic T cell proliferative response (Tables 1 and 2). In contrast, NMA did not affect splenic cell proliferation when added at 100 μg/ml in the absence of fumonisin B_1. Additionally, fumonisin B_1 (12.5–50 μg/ml) significantly ($p < 0.01$) stimulated [^{3}H]-thymidine incorporation in the fibroblast-like cell line L929 (data not shown), similar to the proliferative results reported following incubation of confluent cultures of Swiss 3T3 fibroblasts with fumonisin B_1 (Schroeder et al., 1994).

In conclusion, elicitation of NO production suggests that induction of iNOS can be considered a previously unrecognized effect of fumonisin B_1 exposure and that NO may be responsible for certain of the in vivo effects observed. This study also shows that in the absence of NO production fumonisin B_1 enhances T cell proliferation. Thus, fumonisin B_1 may function in vivo to activate the immune system by two separate and distinct means: potentiation of lymphoproliferation and stimulation of NO production by macrophages. This might have clinical relevance against infectious diseases and cancer.

Acknowledgements

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